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Introduction

1.1 A definition

Morphogenesis means the beginnings of form and, in the context of biological development, is an ambiguous word: the term may refer either to the structural changes that we observe as embryogenesis proceeds or to the underlying mechanisms that are responsible for them. Provided that we acknowledge these two facets, we can accept the ambiguity and let the context define the meaning. The important aspect of the word is *change*: morphogenesis is the study of how biological form changes, usually to become more complex, and its domain extends across the living world.

Morphogenesis is the most obvious process of development because it is from their structures that we recognise organs and organisms. It is also the most complex because the genesis of form requires the dynamic coordination of the various activities of a great many cells. To make matters worse, the processes of organogenesis tend to take place inside opaque embryos so that it is usually impossible to observe the events directly. Most morphogenetic research has therefore focussed either on describing the stages of organogenesis using fixed tissue or on showing how the properties of particular cells and the molecules that they synthesise can play a role in tissue formation. Relatively little attention has been paid to integrating the mix of molecular, cellular, tissue and dynamic properties that underly organogenesis.

One reason for this lack of attention is that, because the generation of morphology is poorly understood at the genetic level, many biologists believe that we do not yet have sufficient information to elucidate the principles underlying morphogenesis (e.g. Raff & Kaufman, 1983, p.5). It is true that our understanding of both the genomic and the molecular basis of cell behaviour is limited and inadequate, but this truth is, in my view, thoroughly irrelevant. Using it as an excuse for not trying to understand how cells exercise their properties to generate structure is much like saying that we should not study molecular biology because the quantum mechanical equations governing the interactions between nucleic acid bases have not been solved exactly. As our ignorance of the detailed solutions to

these equations has not inhibited progress in molecular biology, so our ignorance of the genetic basis of cell behaviour need not inhibit us from seeking to investigate, for example, the molecular and cellular mechanisms that cause mesenchymal cells to form bones and the general principles responsible for their diversity of form.

The belief that questions at one level of complexity cannot be answered until underlying problems have been solved is an example of the *reductionist* fallacy. This is so because the belief assumes that, were the underlying problems solved, the solutions would allow the prediction of the answers to the higher-level questions. In fact, there will always be higher-level truths that could not have been predicted from the lower-level ones (one cannot predict the properties of water from quantum mechanics or the behaviour of a virus from its DNA sequence) and, indeed, it is often hard even to understand these higher-level truths in terms of lower-level ones because the interactions can be extremely complex (Tennent, 1986). The restriction that our ignorance of genetic detail imposes on the study of morphogenesis is that the language of molecular biology cannot in general be used to explain the development of form; instead, we must use that of cell phenomenology. This done, we must wait for molecular biologists to provide the details of the genomic interactions that underpin these cellular events.[1]

I do not want to let the reader think that he or she is about to be given a complete phenomenological analysis of morphogenesis, but it is as well to be clear about the types of problems and solutions that will be dealt with here. The book starts from the simple premise that two main classes of event take place in cells during embryogenesis: making decisions and executing them. In the decision-making process, called pattern formation because it is responsible for determining the patterns of cell differentiation that will arise in the embryo (Wolpert, 1969), cells respond to position-dependent signals either picked up in their environment or resulting from their developmental history. During the executive processes, cells respond to these signals by synthesising new substances or changing their properties. Some of these changes may in turn lead to cell reorganisation and the generation of new structures and it is on these that morphogenesis focusses. This picture is of course highly idealised as it is only in a very few cases that a single stimulus and an immediate response are sufficient to specify organogenesis. In most cases, the structural changes that take place depend on how these new properties interact with the existing environment and may also require more than a single instructional cue.

[1] A direct parallel holds in physics: thermodynamics was invented in the nineteenth century to explain a range of thermal and energetic problems, with the solutions being based on such macroscopic properties as heat and free energy. An understanding of what these properties actually mean at the atomic level had to await the invention of statistical mechanics in the early part of this century.

In the following pages, we will explore how changes in cell properties and behaviours lead to relatively simple changes in tissue structure. Our concern will be to study the process of morphogenesis and we will generally ignore questions about how cells acquire new properties and how tissues become functional. The former is part of the pattern-formation scheme and is still not understood although it has been extensively studied (for review, see Slack, 1983). As to tissue function, it usually plays no role in the early stages of morphogenesis (see Weiss, 1939) and it is only after a structure has been formed that its function becomes important. There is therefore no conceptual problem in studying morphogenesis in isolation.

1.2 The approach

There are three ways in which a study on morphogenesis might be ordered: by a single underlying theme, by system or by mechanism. There is no single unifying theme underlying morphogenesis, while the range of systems that have been studied in this context is too diverse to sustain a coherent organisation; by default, therefore, this book is mainly ordered by mechanisms, although they are of course grouped. I have, however, tried to discuss at one point or another most of the major tissues that have been investigated,[2] although, because morphogenesis normally involves more than one property, the mechanism under which a particular system has been discussed is sometimes arbitrary. As to the mechanisms, it has generally been agreed by all developmental biologists from Roux (e.g. 1895) and Davenport (1895) onwards that relatively few are required to generate tissue organisation, even if we do not know exactly how they lead to the formation of most structures. While an elucidation of these mechanisms forms the major part of the book, there is an accompanying theme: if we are to explain how tissue organisation is laid down, we also have to understand the interactions between the cells and the environment in which they operate.

The range of cell and molecular mechanisms underpinning morphogenesis is very wide: some are dynamic (e.g. epithelial invagination), others are more static (e.g. changes in cell adhesion). Some involve cells acting as individuals (e.g. fibroblast movement), others require cellular cooperation (e.g. the formation of condensations). The environments in which cellular activity takes place include both other cells and extracellular matrices, as well as the macroscopic boundaries that constrain cell activity. As to the interactions among the cells participating in the morphogenetic enterprise, some initiate the process, others coordinate the activities of large numbers of cells and generate the physical forces that lead in turn to structural change. Finally, there are interactions which constrain these forces and activities and so eventually stabilise the newly formed structure.

[2] The major exception is the morphogenesis of the nervous system.

The central feature of the approach here is to focus on the processes and mechanism by which cellular organisation emerges in embryos with a view to explaining how the interactions between the cells and their environment lead to the formation of new structures. The reader might think that looking for explanations at the cellular level, even if they are a little more complex than usually considered, is only stating the obvious, because tissues are made from cells. The cell is not, however, merely the unit of tissue construction, it is also the unit of genomic expression and, hence, reflects the scale at which genetic mechanisms give rise to new phenotypes. These intracellular molecular changes lead to the cell's acquiring new properties which, in turn, generate structural changes at the multicellular level; fortunately, there is usually little need to know the details of the molecular mechanisms in order to understand how these new properties work. To pick up the point made earlier, there are not only philosophical reasons for not worrying about our ignorance of the molecular basis of morphogenesis, there are also practical ones.

The reader will soon note that this is a book that concentrates on the developmental phenotype and pays relatively little attention to the current exciting work on the genomic basis of embryogenesis. This is not because I think such work unimportant, but because it does not, as yet, provide helpful perceptions on morphogenesis. It should, and it probably will, but not until morphogenetic phenomena have been described that are sufficiently robust and well-defined to lend themselves to analysis using the wide range of DNA-based technologies now available. I hope that the reader will be able to note those phenomena described in the following pages that will be appropriate for analysis by such techniques and, equally important, those that will not.

There is, however, one aspect of classical molecular biology that I think is helpful in understanding morphogenesis and that is the concept of self-assembly. This explains how protein subunits and viruses assemble on the basis of all the information required for assembly being built into the molecules themselves (for review, see Miller, 1984). I believe that something similar can lead to cells organising themselves into tissues and that, once the decisions on changes in cell properties have been taken, the combination of cell activity and environmental interactions is enough to generate the new structure.[3] If this view is correct, some aspects of cellular morphogenesis are directly analogous to the self-assembly of protein chains to form a functional molecule (e.g. haemoglobin or collagen) or of viral proteins and nucleic acid to form a virus or phage (e.g. tobacco mosaic virus or T4 phage). As there is nothing mysterious or magical about the assembly of

[3] Wilson's classic study (1907) showing that isolated sponge cells will reaggregate and form their original structures is the original example of cellular self-assembly while the sorting-out experiments of Townes & Holtfreter (1955) show that such phenomena occur in vertebrates.

proteins and DNA and we do not have to look for other, unspecified, external 'factors' to direct their morphogenesis, so it is with cellular morphogenesis.

The analogy between molecular self-assembly and tissue morphogenesis brings me to the theme that underpins the last part of the book, that organogenesis requires a dynamic as well as a molecular or cellular basis. In order to understand how cells form a tissue, we require insight into the forces that lead to structural change and the ways that the tissue boundaries constrain these forces as much as we need to know the details of the cell and molecular interactions. We also have to show why a new structure should be stable as much as we have to explain, for example, why cells may start to adhere specifically to a new substratum. In short, we need to know how the pieces of the morphogenetic process, the properties, the environments and the interactions, fit together to give a complete picture of the process of tissue formation. The reader with an interest in physics will note that seeking to understand tissue formation in terms of dynamic properties such as stability, forces and boundary conditions is closely analagous to solving a complex dynamic problem in physics. The use in the last chapter of this semi-formal approach to the interactions responsible for morphogenesis will, I hope, provide some insights into the subject that compliment more traditional descriptions.

1.3 The plan

The book is divided into five main sections with inevitable degrees of overlap in their contents. After this introduction, the first main section (Chapter 2) is intended to provide some useful background: it includes a brief history of the subject and a summary of traditional and contemporary approaches to the study of morphogenesis. Chapter 3 focusses on a few morphogenetic case studies; these have been selected partly because they are quite well understood, partly because they demonstrate the range of problems that need solving and partly because they have interested me. These case studies are used to illustrate the range of problems that students of morphogenesis have to solve and the sorts of solutions that they have found. The next three chapters detail many morphogenetic phenomena and the molecular and cellular properties that generate them; these properties can be viewed as a morphogenetic tool kit (see Appendix 1). Chapter 4 covers the molecular basis of morphogenesis and discusses the roles played here by the extracellular environment, the cell membrane and the intracellular cytoskeleton. Chapters 5 and 6 describe the morphogenetic properties of fibroblasts and epithelia, the two main types of cells found in early embryos, and considers a wide variety of the tissues that they form. The last section seeks to show how the dynamic interactions among cells and their environment play a central role in the processes of tissue

formation and uses the analogy of the differential equation to illuminate the types of process that together lead to the morphogenesis of a stable structure. The section ends with a brief attempt to integrate the cellular basis of morphogenesis with events taking place at the level of the genome.

The reader will soon notice that this book deals only with morphogenesis. I have omitted almost everything that I judged peripheral to this topic: there are no background chapters on descriptive embryology or cell biology and technical details are rarely given. Furthermore, as I wanted to write a book that was short enough to be read easily, I have usually focussed on the major conclusions and the morphogenetic significance of the work that I have cited rather than analyse the experiments on which they were based. As to the mechanisms that underpin morphological change, I have tried in all cases to give examples of how and where they are used, but have not usually attempted to discuss the details of their molecular basis.

My intention has thus been to lay out the major themes of the subject rather than to be comprehensive. The phenomena of morphogenesis extend throughout the living world and the material chosen for a book on the subject has to be more than just interesting to merit inclusion, otherwise the text would be too long to be readable and hence be useless. As to the references, perhaps the most useful part of the book, my policy has been to give key historical articles to the major contributions and to cite sufficient contemporary reviews and papers to guide the reader who would like to pursue his or her own interests further.

2

Background

2.1 The past

A brief survey of the history of embryology shows that attempts to understand the mechanisms responsible for the structures that emerge in embryos have not had the highest priority among what we would now call developmental biologists.[1] Indeed, the preformationist approach that directed much of seventeenth and eighteenth century thinking implicitly denied that there are morphogenetic problems to solve. Nevertheless, the contributions made by scientists interested in how structure emerges in the developing organism have been responsible for redirecting the subject of embryology when it had been lead down blind alleys by scientists who did not trust or want to believe the evidence of their eyes. This chapter starts by reviewing briefly two such blind alleys, preformationism and the biogenetic law, partly to pay homage to some distinguished developmental biologists who changed how we think and partly to provide some background before we consider the strategies that have governed recent research into morphogenesis.

2.1.1 Preformationism

Aristotle and Harvey, the two scientists whose thought dominated embryology until the seventeenth century, both considered that structure arose in the embryo through *epigenesis*. This is the view that most if not all embryological structure emerges after fertilisation and is, with some interesting reservations that we will mention later, the view taken today. The mechanisms by which epigenesis occurred were not speculated upon; instead, it was said that the early embryo had a 'forming virtue'. Needham, in his classic book on the history of embryology (1934) points to Sir Kenelm Digby, who wrote in 1644 and before Harvey, as the first person to state in the context of development that explaining by naming was nonsense and

[1] A recent symposium volume on the history of embryology (cited under Tennent, 1986) pays no attention to the topic; neither *morphogenesis* nor any of its obvious synonyms is even a category in the index!

'the last refuge of ignorant men, who not knowing what to say, and yet presuming to say something, do often fall upon such expressions'. Digby asserted instead that the development of form required a 'complex assemblement of causes' and he was perhaps the first person to realise how very complicated are the processes of development.

Such rational approaches were rare. Needham (1934), Gould (1977) and many others have described how, at the end of the 17th century, an alternative view of development, and one that had been a source of speculation since antiquity, came to dominate the subject. The approach was called *preformationism* and supposed that all structures were initially present as miniatures in the egg. It thus held development to be no more than the differential enlargement or unfolding of existing structures. Needham points to two reasons for the change in paradigm: first, Aristotelian thinking was out of fashion and, second, Marcello Malpighi had found in 1672 that the outlines of embryonic form were present (the embryo had gastrulated) at the earliest stages of chick development that he could observe, which turned out to be after the egg had moved down the oviduct. At about the same time, Swammerdam, after hardening a chrysalis with alcohol, discovered a perfectly formed butterfly within it. He therefore deduced that the butterfly structure was present but masked within the caterpillar (was he so wrong?) and hence within the egg.

At this point, reasonable scientific study was abandoned by many biologists and wish became the father of thought and the grandfather of observation: they claimed to see small but fully formed organisms in the sperm of men, horses, cocks and other animals and also in some eggs. Other scientists failed to see such wonders, but their reservations were ignored. Needham also points out that, because of theological concern about the implications of spontaneous generation, preformation was more acceptable than epigenesis as an explanation of development: if structure, even of lowly animals, could arise *de novo*, then the same events could take place in human development, a conclusion whose theological implications were uncomfortable. Preformationists were quite prepared to take their view to the logical limit, the *emboitement* principle, and say that within each animalcule was a smaller animalcule and within that a smaller one and so on. Thus, in the ovaries of Eve (or the testicles of Adam) was the forerunner of every successive human.

The preformationist approach was shown to be wrong by the observation of a great scientist, Carl Friedrich Wolff: he did not, for complex reasons, believe in preformation and, to disprove it, chose to investigate how blood vessels appeared in the chick. He was able to demonstrate in 1759 that, at the resolution of his microscope, the blood vessels of the chick blastoderm were not initially apparent, but emerged from islands of material surrounded by liquid. Haller, a contemporary, had an immediate and totally dismissive response to this evidence: the blood vessels had been

there all the time but only became visible later. Wolff then found incontrovertible evidence that an important structure would form while being studied. He demonstrated in 1768 that the chick gut was not initially a tube but was formed by the folding of the ventral sheet of the embryo. Needham summed up this result nicely when he wrote that 'it ruined preformation'. It did, however, take a long time to die and Gould (1977), in his analysis of Bonnet's justification of preformationism, explains why. The main reasons were that, as microscopy was poor, much was known to be going on that could not be seen and, as there was then no cell or atomic theory, there were no size limits to constrain speculation. Gould also points out that scientists such as Bonnet were concerned to be scientific rather than vitalistic: as no mechanism for epigenesis could be advanced, it would be irrational and unscientific to believe in it.

These problems do not, at first sight, concern us today for preformation seems dead and buried. Indeed, the reader may think such history entertaining but irrelevant and wonder why it is worth dredging up now. In fact, the preformationist/epigenetic dichotomy is still very much with us, as Baxter (1976) has pointed out, but the problem is phrased rather differently now for we have to replace epigenesis with regulative development and preformation with a predetermined order laid down in the egg. There is even a case for arguing that the *emboitement* principle was a brilliant, if premature, insight into the nature of DNA and the continuity of the germ plasm.

What we would now like to know is whether structure is directly determined by DNA-coded information laid down in the egg (mosaic embryos) or whether it arises later and more indirectly from changes in the properties of the cells and the tissues (regulative embryos). In fact, the answer, which seems first to have been pointed out by Roux (see Oppenheimer, 1967, p.70) and which is not very helpful to the working scientist, is both, and the extent to which either may contribute depends on the animal or the tissue under consideration; some eggs are more mosaic and others more regulative. Only experimentation can demonstrate where in the spectrum a given tissue is to be found and the mechanism by which that structure forms.

The much more interesting morphogenetic problem, for me at least, is considering the extent to which structure can be reduced to instruction. It is important to know in principle whether the fine detail of tissue organisation can be explained in terms of or predicted from the properties of the participating cells and the environment in which they operate or whether a closer control is required. We can start with one of two extreme (and incorrect) views: organogenesis is either a wholly stochastic process based on the interactions of cells with their environment or is predetermined by precise information stored in the genome that cells interpret as specific instructions. At the end of the book, and after the evidence has been

considered, we will examine the extent to which morphogenesis can, in principle at least, be reduced to molecular biology.

2.1.2 The biogenetic law

The second blind alley that I want to touch on is the extraordinary position in which developmental biology found itself at the end of the nineteenth century. The subject was dominated by a biologist called Ernst Haeckel who was not an embryologist. He held that the developmental stages through which an embryo passed as it approached the mature form were a reflection of *adult* evolution and founded a school to investigate the evidence for and the consequences of this approach. The war cry of this school was 'ontogeny recapitulates phylogeny' and it was war, albeit of the verbal variety, that Haeckel declared on anyone who chose to say either that he was wrong or that embryology had any purpose other than to confirm the general validity of this law.[2]

The situation seems all the more ridiculous today when we realise that, fifty or so years earlier, von Baer had shown that the evidence supported the view that the developmental stages through which the embryo of a *higher* animal passed as it matured were a reflection of the embryos, but not the adults, of *lower* animals and hence of its *embryonic* evolution. Gould (1977) points out that the intellectual environment in Germany at that time was receptive to the type of global approach put forward by Haeckel and that, once a model held centre stage, its proponents were awarded all the academic positions and the approach became self-sustaining. Furthermore, counter evidence was not enough to break the hold of the theory: Haeckel could, and did, argue that one or another exception was not enough to negate a theory that held across the whole of the animal kingdom.[3]

If logic, knowledge and observation could not rock the boat, what else was there? The simple answer is a change of fashion: the spell of the biogenetic law was broken when the biological community realised that there were profound developmental problems that the law did not address. Once this step had been taken, the law, Haeckel and his tradition disappeared off the intellectual map in a decade. It was Wilhelm His who pointed the way: he showed that changes in the shape of the the embryo (Fig. 2.1) and the developing gut could be modelled by a rubber tube under complex tensions. Though not at first sight a revolutionary insight, its

[2] Gould (1977) has written a comprehensive review of the controversy, while a pithy summary is given by Raff & Kaufman (1983).

[3] It is not at first sight obvious that a theory would hold the attention of professional scientists just because it had qualities that were philosophically pleasing, particularly when there was contradictory evidence. Gould (1977, p.102) points out that, although the theory was wrong on the grand scale, it could be useful in analysing how specific characteristics could change and hence explain local evolutionary relationships among similar animals and he gives as an example Weismann's analysis of colour patterns in caterpillars (1904).